Test cells
|
Prepare a cDNA library directionally cloned in lambda ZAP II vector
|
Convert into plasmid library by in vivo excision
|
Convert into single stranded cDNA library
|
Anneal to mRNA from reference cells
|
"Fill in" the plasmids with Klenow fragment of E coli DNA polymerase I and Pfu DNA polymerase
|
Digest the heteroduplex loops with RNase I
|
Open the plasmids with S1 nuclease
|
Ligate to the stuffer (Figure 2)
|
Transfect into E.coli cells
|
Plate the library and obtain replicas
|
Screen the library with radiolabeled stuffer
|
Select the positive clones and prepare plasmid DNA
|
Sequence the plasmid DNA with the primers a and b (Figure 2)
|
Analyze the sequence by comparison with homologous genes from database

Figure 1

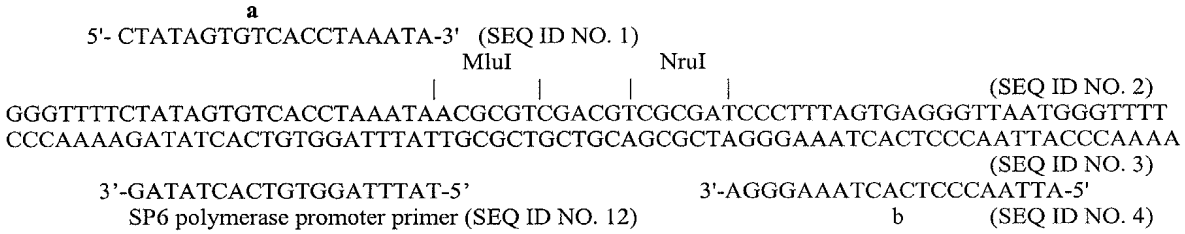
a
5'- CTATAGTGTCACCTAAATA-3' (SEQ ID NO. 1)
MluI
NruI
(SEQ ID NO. 2)
GGGTTTTCTATAGTGTCACCTAAATAACGCGTCGACGTCGCGATCCCTTTAGTGAGGGTTAATGGGTTTT
CCCAAAAGATATCACTGTGGATTTATTGCGCTGCTGCAGCGCTAGGGAAATCACTCCCAATTACCCAAAA
(SEQ ID NO. 3)
3'-GATATCACTGTGGATTTAT-5'
SP6 polymerase promoter primer (SEQ ID NO. 12)
3'-AGGGAAATCACTCCCAATTA-5'
b
(SEQ ID NO. 4)

Figure 2

ATGTGTGGGGTTTTCTA-STUFFER-AATGGGTTTTGATTGAAGCT (SEQ ID NO. 5)

	Ile Cys Ile Glu Ala (SEQ ID NO. 6)
Sequence obtained by SDBC:	ATG TGT G-G ATT GAA GCT (SEQ ID NO. 7)
Sequence obtained by RT PCR from MCF7 cells:	Ile Cys Val Ile Glu Ala (SEQ ID NO. 8) ATG TGT GTG ATT GAA GCT (SEQ ID No. 9)
Sequence obtained by RT PCR from normal breast cells:	Ile Cys Glu Ile Glu Ala (SEQ ID NO. 10) ATG TGT GAG ATT GAA GCT (SEQ ID NO. 11)

Figure 3